STERIS®

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STERIS PROCESS™ BIOLOGICAL INDICATOR KIT 510(K) SUMMARY

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STERIS PROCESS BIOLOGICAL INDICATOR KIT
510(K) SUMMARY

Device Name

- Trade name: STERIS PROCESS Biological Indicator Kit
- Common name: Biological Indicator
- Classification name: 21 CFR 880.2800 Sterilization process indicators
  (a) Biological sterilization process indicator

Predicate Device

UniSpore Biological Indicator (MDT Biologic Company)

Spordex/Spordi Biological Indicators (AMSCO Medical Products Division)

Description of Device

The STERIS PROCESS Biological Monitoring Kit contains the following:

- 20 spore strips. Single species bacterial endospore, *Bacillus stearothermophilus* at a nominal population of 1.0×10^5 cfu/strip, inoculated on a chromatography grade paper strip and contained in a tear-open glassine envelope.
- 20 growth medium tubes. 16 mm x 75 mm screw capped glass vials containing 5 ml of sterile, modified soybean casein digest broth (TSB) and 18 mg/liter phenol red pH indicator.
- Transfer clip. Tensioned plastic clip for use in fixation of the spore strip throughout exposure and transfer of the strip into the media tube.
- Incubator. $56 \pm 2°$ C dry bath incubator, capable of holding up to 10 medium tubes.

Intended Use of Device

The STERIS PROCESS Biological Monitoring Kit is intended for use with STERIS SYSTEM 1™, a liquid chemical sterilization system. The Biological Monitor provides independent confirmation that sterilization conditions were achieved during the STERIS SYSTEM 1 processing cycle.

Comparison to Predicate

The technological characteristics of the STERIS PROCESS Biological Monitoring kit are the same as those of the predicate devices. As with the predicate, each spore strip consists of a chromatography grade paper strip that has been impregnated with a known population of a bacterial endospore. Each is packaged in a glassine envelope.

Unlike the predicate, the STERIS PROCESS Biological Monitoring kit has been validated for use in biologically monitoring only the STERIS PROCESS; it has not been qualified for use in monitoring alternative methodologies (e.g., steam, dry heat, ethylene oxide, or

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other liquid processes). Monitoring of a liquid process requires that the spore strip be removed from the glassine envelope prior to processing, to permit the strip to be directly exposed to the sterilant solution. If not removed, the wetted glassine envelope could make removal and aseptic transfer of the strip cumbersome.

Performance Data

As described below, scientific studies were conducted to validate the safety and efficacy of the STERIS PROCESS Biological Monitoring kit. Both *in vitro* (i.e., in a test tube or beaker) and *in situ* (i.e., in a SYSTEM 1 Processor) tests were conducted to evaluate the performance of the device. Clinical tests were not required, as the non-clinical studies that were conducted utilized standard SYSTEM 1 processing cycles which are identical to those which are employed clinically. The following sections summarize the test methods and results for each set of tests.

Comparative resistance of organisms

Table 1
Comparative Resistance of *B. stearothermophilus* and *B. subtilis* to the STERIS PROCESS

Spore Inoculum and Population	Exposure Time (seconds)	Concentration (ppm)	Sterile Strips	Tested Strips	D Value (seconds)
B. stearothermophilus (1.9×10^6 cfu / strip)*	60	2,000	5	10	9.3
	90	1,000	5	30	14.9
	120	1,000	25	30	17.1
B. subtilis var. *niger* (4.7×10^6 cfu / strip)*	40	1,000	17	50	6.0
	45	1,000	21	30	6.3

*Manufacturer's assayed population

Bacillus stearothermophilus and *Bacillus subtilis* spores are commonly used as indicator organisms for monitoring sterilization processes. STERIS conducted testing of spore strips containing each of these organisms to determine their respective resistance to the STERIS PROCESS.

In vitro testing was conducted at 50°C, which is the minimum temperature specified for sterilization in STERIS SYSTEM 1. Spore strips were tested using the standard peracetic acid concentration present in the STERIS PROCESS (2000 ppm), and also at one half the standard concentration (1000 ppm).

For each series of tests, a quantity of spore strips were removed from their glassine envelopes and exposed to the sterilant solution for a predetermined exposure time. Immediately after exposure, each strip was placed into growth media and incubated at 56°C (*B. stearothermophilus*) or 37°C (*B. subtilis*) for up to seven days. Culture media was examined at 24 hour intervals, and positive or negative growth was recorded. Decimal reduction times (i.e., D-values) were then calculated based on partial positive data using the Stumbo method, as shown in the following equation:

$$D = \text{Decimal reduction time} = \frac{\text{Time of exposure}}{\text{Log of initial population} - \text{Log}\,(2.303 \times \text{Log } r/q)}$$

where r = the number of strips exposed and
q = the number of strips that were sterile

The results of this testing are shown in Table 1 above. *B. stearothermophilus* was found to be more resistant to the STERIS PROCESS than *B. subtilis*, and therefore presents a more rigorous challenge. The D value for *B. stearothermophilus* was found to be 9.3 seconds using the standard concentration of the active ingredient for the STERIS PROCESS, and 17.1 seconds when using one half the standard concentration. D values for *B. subtilis* were slightly shorter.

Note that the standard sterilant exposure period for the STERIS PROCESS is 12 minutes or 720 seconds. A D value of 9.3 seconds equates to greater than a 77 log reduction during the standard STERIS PROCESS cycle. This greatly exceeds the 12 D period which is generally recognized as the minimum requirement for sterilizers.

Testing of multiple spore strip lots

Table 2
Variation of *B. stearothermophilus* Resistance to STERIS 20 Sterilant and Steam

Spore Strip Lot	Population[1] (cfu / strip)	# Sterile Strips / # Strips Tested at Exposure Time (seconds)			D Value	
		30	65	105	STERIS PROCESS[2] (seconds)	Steam at 121° C[3] (minutes)
A	1.9×10^5	0/30	19/30	30/30	11.6	2.5
B	1.3×10^5	0/30	27/30	30/30	10.6	1.4
C	1.2×10^5	0/30	29/30	30/30	9.9	2.0
D	3.6×10^5	0/30	9/30	30/30	11.9	1.7
E	2.1×10^5	0/30	27/30	30/30	10.3	2.4
F	1.4×10^5	0/30	3/30	30/30	13.6	1.6
G	1.3×10^5	0/30	8/30	30/30	13.0	2.0
H	1.8×10^5	0/30	29/30	30/30	9.7	3.6
I	2.8×10^5	0/30	22/30	30/30	10.9	1.9
Means:	$1.9 \pm 0.8 \times 10^5$	0/30	19 ± 9.5 /30	30/30	11.3 ± 1.3	2.1 ± 0.6

[1] Manufacturer's assayed population
[2] Testing performed at 50°C with one half standard peracetic acid concentration (1000 ppm)
[3] Manufacturer's stated D value

In order to fully characterize resistance of the spore strips used in the STERIS PROCESS Biological Monitoring Kit and ensure that resistance was reproducible, STERIS conducted testing using 9 spore strip lots. Each test was conducted at 50°C using one half of the standard concentration of active ingredient present in the STERIS PROCESS (1000 ppm). This reduced concentration was used due to the very rapid rate of kill of STERIS sterilant. By conducting testing at one half standard concentration, survival times are increased and potential sample error is reduced (i.e., error encountered due to the time needed to remove the sample and provide dilution in growth media).

A total of 90 strips from each lot were tested. Groups of 30 strips were exposed to the sterilant for 30, 65, or 105 seconds. Immediately after exposure, each strip was placed into growth media, incubated at 56°C, and observed daily for growth for up to seven days.

Cultures producing turbidity and/or a color change in the media from red to yellow were interpreted as "positive;" cultures exhibiting no change in color of the media were interpreted as "negative" or sterile.

As shown in Table 2, no strips were sterile after 30 seconds and all strips were sterile after 105 seconds. Testing at 65 seconds of sterilant exposure yielded partial positive data (i.e., some strips were sterile and some were not), from which D values could be calculated using the Stumbo method described in the preceding section.

The mean D value determined for the 9 lots of spore strips that were tested was 11.3 seconds for the STERIS PROCESS (using 1/2 the normal level of active ingredient), with a standard deviation of 1.3 seconds. Note that this compares to the standard STERIS PROCESS cycle time of 720 seconds. Actual D-values during a standard cycle can be expected to be shorter than those shown, as a higher peracetic acid concentration is present.

Recovery validation

Table 3
Bacillus stearothermophilus Recovery Validation

Test Condition	Trial	Results (positive tubes / tested tubes)	
		24 Hours	48 Hours
Vials inoculated with ≤ 10 cfu of the test organism to which a processed strip was added.	1	10/10	10/10
	2	10/10	10/10
	3	10/10	10/10
Controls: vials inoculated with ≤ 10 cfu of test organism without the addition of a processed strip	1	10/10	10/10
	2	8/10	10/10
	3	7/10	10/10

The methodology employed for the *in vitro* studies presented in Tables 1 and 2 was qualified as to any inhibition of spore growth attributable to the presence of sterilant on the spore strips that may be carried into the growth media. Testing was conducted to validate that sterilant carryover was not inhibitory to the expression of outgrowth that would be present due to small numbers of *B. stearothermophilus* spores.

Thirty culture media vials were inoculated with a maximum of 10 cfu of *B. stearothermophilus* spores. Into each vial was added a spore strip immediately after it had been exposed to sterilant use dilution for 12 minutes at 50°C. Thirty positive controls were prepared in which identically inoculated vials did not have a processed strip added. Negative controls to demonstrate the sterility of the exposed spore strips were also conducted in which processed strips were added to media vials that were not previously inoculated (data not shown). All vials were incubated at 56°C, with results recorded at 24 hour intervals.

As shown in Table 3, all tubes that had been inoculated with low numbers of the test organism demonstrated spore outgrowth within 48 hours. The addition of processed spore strips, with any associated carryover of sterilant, did not adversely affect outgrowth time. The delayed outgrowth at 24 hours in trials 2 and 3 of the positive controls is consistent with low inoculum, and the time that may be required for what could be only 1-2 spores to sufficiently propagate so that turbidity can be observed.

Instructions for the STERIS PROCESS Biological Monitoring Kit indicate that incubation should take place for up to seven days. This testing, therefore, demonstrates that the culture media and incubation method provided with the STERIS PROCESS Biological Monitoring Kit is capable of indicating the presence of the test organism, even when it is present in very small quantities. Furthermore, this testing demonstrates that any sterilant carryover to the growth media is not inhibitory to spore outgrowth. This result is to be expected, given that the half life of STERIS sterilant at its use dilution is approximately 20 minutes. Any sterilant carried by the spore strip into the media could be expected to become inactive long before spore germination and outgrowth occurs.

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Processor validation: sterilization during full cycles

Table 4
Sterilization of Spore Strips by the STERIS PROCESS
Using 12 Minute Sterilant Exposure (Standard Processing Cycles)

Population of *B. stearothermophilus* (cfu / strip)*	Exposure Time (minutes)	Processed Strips (Sterilized Strips / Tested Strips)	Control Strips (Sterilized Strips / Tested Strips)
2.4×10^5	12	50/50	0 / 20
1.9×10^6	12	100 / 100	0 / 20
1.9×10^5	12	20 / 20	
1.3×10^5	12	10 / 10	
1.2×10^5	12	10 / 10	
3.6×10^5	12	10 / 10	0 / 10
2.1×10^5	12	10 / 10	
1.4×10^5	12	10 / 10	
1.3×10^5	12	10 / 10	
1.8×10^5	12	10 / 10	0 / 10
2.8×10^5	12	10 / 10	
Totals:	12 minutes	250 / 250	0 / 60

* Manufacturer's assayed population

Data characterizing the ability of the STERIS PROCESS to inactivate STERIS PROCESS Biological Monitoring spore strips at the standard exposure time of 12 minutes are presented in Table 4. In 250 trials, under standard conditions for use, the STERIS PROCESS Biological Monitors (*Bacillus stearothermophilus* spore strips) were reproducibly sterilized during the regular SYSTEM 1 processing cycle.

A total of 60 *in situ* control cycles were conducted in which the active ingredient was omitted from the sterilant. None of these cycles resulted in sterilization of the spore strips, thereby indicating that neither the sterilant inert ingredients nor the four subsequent sterile water rinses were responsible for sterilization of spore strips.

Processor validation: half cycle testing (demonstration of overkill)

Table 5
Sterilization of Spore Strips by the STERIS PROCESS
Using 6 Minute Sterilant Exposure (Half Cycle)

Population of *B. stearothermophilus* (cfu / strip)*	Exposure Time (minutes)	Processed Strips (Sterilized Strips / Tested Strips)	Control Strips (Sterilized Strips / Tested Strips)
2.4×10^5	6	50 / 50	0 / 20
1.9×10^6	6	100 / 100	0 / 20
1.9×10^5	6	20 / 20	
1.3×10^5	6	10 / 10	
1.2×10^5	6	10 / 10	
3.6×10^5	6	10 / 10	
2.1×10^5	6	10 / 10	
1.4×10^5	6	10 / 10	0 / 10
1.3×10^5	6	10 / 10	0 / 10
1.8×10^5	6	10 / 10	
2.8×10^5	6	10 / 10	
Totals:	6 minutes	250 / 250	0 / 60

* Manufacturer's assayed population

A widely accepted strategy for validating a sterilization cycles is to show that the cycle has demonstrable overkill. One method for demonstrating this is to evaluate the effectiveness of a sterilization cycle which is shorter than the standard cycle, thereby showing that a full cycle has significant overkill. The degree of overkill can be estimated based on an extrapolation of the partial cycle results.

STERIS demonstrated the overkill present in the STERIS PROCESS by conducting testing using SYSTEM 1 Processors that were specially modified to have a 6 minute sterilant exposure -- one half of the normal 12 minute exposure. As shown in Table 5, *Bacillus stearothermophilus* spore strips were reproducibly sterilized in 250 out of 250 trials conducted using SYSTEM 1 Processors that were modified to provide one half exposure.

The initial spore populations present on the spore strips used in this testing ranged from 1.2×10^5 to 1.9×10^6. This testing, therefore, demonstrated at least 5.08 to 6.28 log reduction during cycles which are one half of the standard 12 minutes. Extrapolating these results indicates that full STERIS PROCESS cycles would be expected to achieve a minimum of a 10.2-12.6 log reduction of *Bacillus stearothermophilus* spores.

It should be noted that the method used for this testing allows only a very conservative estimate of the total lethality of the STERIS PROCESS. Since no viable spores remained on any of the 250 strips after 6 minute exposure, D values cannot be calculated, and only a relative statement can be made about the rate of kill (i.e., the D value must be less than 6 minutes / 6.28 logs or .96 minutes, although this testing cannot define how much less). Quantitative D value data presented in Table 1 show that D values are 9.3 seconds, which equates to a 77 log reduction during the 12 minute standard cycle.

In situ controls were also conducted using processors modified to provide a 6 minute sterilant exposure. These control cycles omitted the active ingredient of the STERIS 20 sterilant. None of these controls cycles resulted in sterilization of the spore strips, thereby demonstrating that neither the sterilant inert ingredients nor the four subsequent sterile water rinses were responsible for sterilization of spore strips.

Validation of spore adherence -- In vitro determination

Table 6
In Vitro Determination of Adherence of *B. stearothermophilus* Spores
to the Spore Strip Carrier

	Strip Number	Spores Removed (cfu / strip)
Assayed Initial Population (cfu / strip) 1.7×10^5	1	130
	2	400
	3	200
	4	330
	5	430
	Mean: 298	
	% Removed: 0.2%	

Unlike steam and ethylene oxide sterilization processes, the STERIS PROCESS employs a liquid chemical agent to achieve sterilization. In addition to the positive control testing shown in Tables 4 and 5, STERIS conducted additional testing to validate that the liquid

chemical STERIS PROCESS did not have a significant effect on the challenge presented by the spore strip. This testing included an evaluation of the spore populations on strips that were processed in normal SYSTEM 1 processing cycles in which the active sterilizing agent was omitted, and *in vitro* tests which sought to quantify the number of spores that could be removed from a strip by a liquid process.

To assess the removal of spores from a spore strip, strips were subjected to STERIS 20 Sterilant use dilution which omitted the active ingredient (peracetic acid). Each strip was immersed in 100 ml of the dilution for 30 minutes at 50°C. The solution was stirred for one minute prior to sampling to encourage disassociation of spores from the strip. After exposure, the strip was removed from the solution, and the number of spores in solution was determined using standard TSA plate count methods.

The 30 minute exposure period used for this testing is longer than the 12 minute sterilant exposure that occurs during the standard SYSTEM 1 processing cycle. Exposure time is also much greater than the time required to produce total spore kill, as shown in Table 2. Even under this extreme condition, the percentage of spore population removed is well within the expected population variation present on spore strips, and has a minimal effect on the population remaining on the spore strip (e.g., $1.7 \times 10^5 - 3.0 \times 10^2 \approx 1.7 \times 10^5$). Similar results were obtained when testing strips using *B. subtilis* (data not provided).

It should be noted that any spores which are removed from the spore strip carrier would be subject to the same sterilizing conditions as the strip itself. Whether spores are inactivated on the carrier or in solution is immaterial, provided that the spores remaining on the spore strip are sufficient in number to present a valid challenge.

Validation of spore adherence -- in situ determination

Table 7
In Situ Determination of Adherence of *B. stearothermophilus* Spores to the Spore Strip Carrier

Site	Spore Strip Populations, mean ± std. dev. ($x10^4$ cfu per strip) Lot 1	Lot 2	Lot 3
GPC 1	9.0 ± 1.8	10.3 ± 2.0	9.4 ± .9
GPC 2	9.4 ± 2.6	10.7 ± 1.9	9.1 ± 2.6
GPC 3	8.9 ± 1.8	11.0 ± 2.0	7.6 ± 2.2
GPC 4	9.8 ± 2.9	10.7 ± 2.2	8.2 ± 2.7
GPC 5	9.9 ± 2.0	10.7 ± 2.6	8.1 ± 1.3
GPC 6	8.9 ± 1.3	10.1 ± 1.9	8.3 ± 1.3
GPC 7	9.5 ± 1.8	10.4 ± 1.3	8.1 ± 1.7
GPC 8	9.7 ± 1.2	10.3 ± 2.2	8.3 ± 1.8
GPC 9	9.3 ± 2.0	10.4 ± 1.5	8.0 ± 1.5
GPC 10	8.9 ± 1.5	9.9 ± 1.3	8.5 ± 2.1
GPC Mean	9.3 ± 1.9	10.5 ± 1.9	8.4 ± 1.9
Flex 1	10.9 ± 1.8	11.0 ± 2.0	10.8 ± 1.4
Flex 2	10.3 ± 2.0	11.7 ± 2.0	10.6 ± 2.3
Flex 3	11.9 ± 2.4	10.6 ± 3.0	10.5 ± 1.8
Flex 4	11.0 ± 2.0	10.3 ± 1.7	9.6 ± 2.9
Flex 5	12.3 ± 1.7	12.4 ± 3.0	11.0 ± 2.2
Flex 6	10.6 ± 1.7	10.3 ± 1.1	10.2 ± 2.2
Flex 7	10.6 ± 2.0	11.0 ± 1.6	10.2 ± 1.1
Flex 8	11.2 ± 1.5	11.2 ± 2.1	9.9 ± 1.5
Flex 9	11.5 ± 2.5	11.2 ± 1.2	9.9 ± 1.4
Flex 10	10.2 ± 1.7	11.0 ± 2.0	9.6 ± 1.8
Flex Mean	11.0 ± 2.0	11.1 ± 2.1	10.2 ± 1.9
Overall Mean	10.2 ± 2.1	10.8 ± 2.0	9.3 ± 2.1
Unprocessed mean	10.0 ± 2.7	12.8 ± 4.5	9.3 ± 3.5

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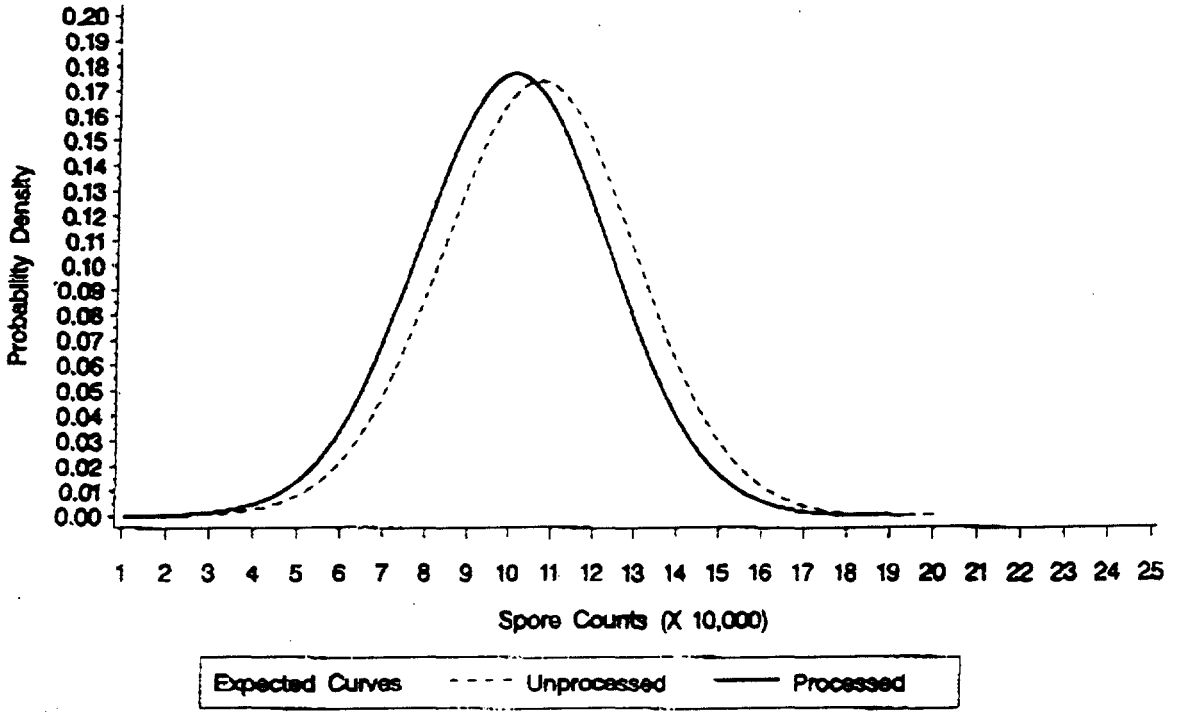
Figure 1
Distribution of Spore Strip Populations on Processed and Unprocessed Strips



In situ testing was conducted to validate spore strip populations after strips had been subjected to actual SYSTEM 1 processing cycles from which the active ingredient was omitted. A total of 600 strips from three spore strip lots were tested, along with an additional control sample of 30 strips. For each test, 10 strips were secured at locations throughout either the SYSTEM 1 General Processing Container (GPC) or Flexible Processing Tray (Flex) and a standard SYSTEM 1 cycle (without peracetic acid) was completed This test was repeated 10 times for each tray and spore strip lot. After processing, each strip was macerated in TSB, sampled, diluted, plated, incubated and counted to determine the spore strip population. The populations were statistically compared to the populations of 10 control samples for each lot which were determined using an identical methodology. Table 7 contains summary statistics for processed and unprocessed strips. Figure 1 shows the population distribution.

An analysis of variance was performed on the test data. Site within the processor was found to not significantly affect post-processing population, thereby confirming that the location within the tray or container does not affect adherence of spores to the spore strip. This suggests that any differences in turbulence of the sterilant within the tray or container does not affect the challenge presented by the spore strip.

STERIS PROCESS BIOLOGICAL INDICATOR KIT
510(K) SUMMARY

The mean population of processed strips was 1.01×10^5, with a standard deviation of 2.15×10^4. The mean population of control strips was 1.07×10^5 with a standard deviation of 3.85×10^4. A statistical analysis of the data confirmed that at a 99% confidence interval of the mean, points 3.9 standard deviations below the mean still exceed a population of 10^4. While the current USP does not contain a section specifically addressing BIs for monitoring a liquid process, USP requirements for BIs used to monitor dry heat, steam, and ethylene oxide all require a minimum population of 10^4 spores per carrier.

Conclusion

In vitro and *in situ* testing has demonstrated that the STERIS PROCESS Biological Indicator Kit is a safe and effective device when used in accordance with the intended use of providing independent confirmation that sterilization conditions were achieved during the STERIS SYSTEM 1 processing cycle.

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